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14. ABSTRACT

Prostate cancer results from complex interactions among genetic, endocrine, and environmental factors. Understanding genetic risk factors that contribute to the occurrence of prostate cancer is crucial to design both preventative and therapeutic strategies and to identify at-risk individuals. This knowledge could reduce the incidence of and death from this disease. The primary objective of this grant is to investigate changes in genes that directly and indirectly regulate levels of male hormones, which in turn, affect prostate cell growth, and may ultimately cause cancer. In this past 8 months, we have genotyped the DNA samples for eight haplotype-tagging SNPs in *IGF1*, an additional ten SNPs in *IGF1R*,one additional SNP in *IRS1* and three additional SNPs in *IGFBP3*, as well as performed genotyping of the *SRD5A2* V89L and *CYP3A4* B1 polymorphisms from the original proposal. We have performed statistical analysis of the 18 IGF1R SNPs and the PI3KCB and SHC1 SNPs. Two of the IGF1R SNPs were significantly associatedwith Gleason score. More analyses are in progress.

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Table of Contents

Annual Progress Report
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INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer in men and the most common cause of cancer mortality in the United States (American Cancer Society, 2006). The American Cancer Society estimates that, during 2006, approximately 234,460 new cases of prostate cancer will be diagnosed in the United States. One in six men will be diagnosed with prostate cancer over the course of a lifetime. In our aging population, research leading to a reduction in the incidence of and mortality from prostate cancer is an urgent necessity. A critical problem in prostate cancer is an understanding of risk factors involved in disease development and aggressiveness. Clinically important genetic risk factors that may result in differences in individual susceptibility to prostate cancer likely include genes involved in androgen biosynthesis, metabolism and regulation and in prostate cell growth and death (Papatsoris et al., 2005; Schaid, 2004). We propose to answer the following questions. What are some of the genetic risk factors that determine who develops prostate cancer? Of those individuals who develop cancer, what risk factors contribute to the age at diagnosis and to aggressiveness of the disease? Using a case-control design, we are testing the hypothesis that common genetic polymorphisms (variants) in genes directly and indirectly involved in altering hormonal levels and prostate cell growth are associated with prostate cancer risk. We are investigating their associations with occurrence of prostate cancer, age at diagnosis, and aggressiveness of the disease as measured by Gleason score and tumor stage-related variables.

BODY

Our progress is described by Tasks. We have been granted a second no-cost extension to complete the statistical analyses and to genotype and analyze additional SNPs in genes that are either in the IGF pathway or that have been described in the literature. We are studying 199 prostate cancer cases and 254 age-matched controls. Data on family history, age at diagnosis, and clinical and pathological characteristics have been obtained for the prostate cancer cases. We are genotyping for the genetic variants in DNA samples from this set of cases and controls.

Aim 1: To assay samples for the genetic variants (genotyping).

Task 1: Design allele-specific primers for genotyping. Test and optimize the genetic assays. Compare with published protocol results. Sequence to confirm that detecting the appropriate alleles. COMPLETED.

We are performing the single nucleotide polymorphism (SNP) genotyping using the MGB Taqman exonuclease (Applied Biosystems) and the MGB Eclipse probe (Epoch Biosciences) assays. We have completed all assay designs including assays for 4 SNPs in *IGFBP1*, 4 SNPs in *IGFBP3*, 8 SNPs in *IGF1*, 18 SNPs in *IGF1R*, 3 SNPs in *IRS1*, 4 SNPs in *SHBG*, and single SNPs for *PI3KCB* -359 T>C, *SHC1* M300V, *SRD5A2* V89L, and *CYP3A4* 392 A>G. For design of the assays, we first submitted sequence encompassing the SNP to the Applied Biosystems (ABI) Assay-by-Design service for designing the assays. For those that ABI could not design, we sent sequence to Epoch Biosciences. They use the MGB Eclipse probe assay, which has a very different probe design than the Taqman assay. The majority of SNPs that could not be designed by Assay-by-Design were able to be designed by Epoch. We then tested and optimized the assay when we received it using known homozygotes and heterozygotes for the variants. For the *IGFBP3* -202 A>C SNP, neither assay could be designed so we performed a restriction endonuclease assay.

Task 2: Screen for variants in insulin-like growth factor binding protein I (*IGFBP-1*) to identify a variant(s) for genotyping. COMPLETED PREVIOUSLY.

We identified 19 variants of which 4 SNPs are required to tag the variation in this gene in Caucasians. These four haplotype-tagging SNPs were used for genotyping in Task 5.

Task 3: Identify male controls which match prostate cancer cases. COMPLETED PREVIOUSLY.

For the 199 prostate cancer cases for whom we have DNA and diagnosis and follow-up data, we have identified 254 age-matched male population-based controls. Dr. Brothman was no longer able to enroll participants in his study, so there are no additional cases or controls available for this study.

Task 4: Aliquot DNA from all samples available. COMPLETED PREVIOUSLY.

However, in this past year, we were running low on 60 of the DNA samples. Therefore, we performed whole genome amplification on those samples using a kit from Amersham in order to increase the amount of DNA. We had previously validated that the DNA after whole genome amplification provided the same results as the non-amplified DNA.

Task 5: Perform genotyping. COMPLETED

The total DNA samples available for genotyping are 453, 199 prostate cancer cases and 254 controls. During previous funding periods, we had completed genotyping on the 199 prostate cancer cases and 254 controls for the following polymorphisms: *CYP17-MspI, IGF-1* STR*, CYP11A* STR*, VDR-BsmI, VDR-Taq1, VDR-polyA, IRS1* G972R*, SHBGI* D327N*, SRD5A2-str*, the *INS* +1127 Ins-PstI*, IRS2* G1079D, PI3KCB, and SHC1 SNPs. We also performed genotyping for four haplotype-tagging SNPs in *IGFBP1*, four in *SHBG,* 8 in *IGF1R,* 3 in *IRS1*, During this past period, we genotyped the samples for the *PI3KCB* -359T/C and the *SHC1* M300V variants, genotyped 2 additional SNPs in *IRS1*, genotyped one of the *IGFBP3* SNPs, and 8 of the *IGF1R* SNPs.

Since the last annual report, we have genotyped the DNA samples for eight haplotype-tagging SNPs in *IGF1*, an additional ten SNPs in *IGF1R*, one additional SNP in *IRS1* and three additional SNPs in *IGFBP3*, as well as performed genotyping of the *SRD5A2* V89L and *CYP3A4* B1 polymorphisms from the original proposal. The lab technician is currently redoing genotyping for samples that did not provide results for individual SNPs. For each SNP, the lab technician has determined which samples have no data, has individually pulled those samples, and then genotyped the selected SNP.

Note: In a previous report, we described that we had decided that it was not useful to genotype the microsatellite repeat markers in *HSD3B2* and *HSD17B2*, as there was no indication that they would be related to function.

Task 6: Read genotypes and enter into our Sybase database.

Genotypes that have been completed are currently entered in an excel spreadsheets. All genotypes generated in Task 5 have been entered into spreadsheets for analysis in Aim 2.

Aim 2: To statistically analyze the association of genes assayed in Aim 1 with prostate cancer age at diagnosis and aggressiveness, as measured by Gleason score and tumor stage-related variables. Aim 3: To statistically analyze the association of genes assayed from Aim 1 with occurrence of prostate cancer.

Task 7: Design data entry forms for entering data into Sybase. COMPLETED PREVIOUSLY, BUT NOT USING.

This task was completed so that we can download the data into Sybase. However, since it is a finite amount of data, it is better to use Excel spreadsheets that are uploaded for analysis with the statistical package SAS. Therefore, we are not using Sybase for storing the data.

Task 8: Edit data. Add data from medical records and Utah Cancer Registry. COMPLETED PREVIOUSLY.

The prostate cancer cases were diagnosed from 1992-2000. Age at diagnosis ranged from 45-78 years with a mean age of 62.6 years and a median age of 63 years. Of the tumors, 10 were well-differentiated, 139 were moderately differentiated and 50 were poorly differentiated. Thirteen of the cases had another type of cancer, either previous to or after diagnosis of prostate cancer. We obtained follow-up data on these cases with the dates of last follow-up ranging from 2000-2002. These data are in the excel spreadsheet with the genotypes. Of the 199 prostate cancer cases, 15 are deceased including 1 case diagnosed at 49 years of age who died from metastatic prostate cancer.

Task 9: Months 25-27: Test models and analysis methodologies. COMPLETED.

Gleason scores are being placed into groupings commonly used in clinical prognosis. Group 1 is Gleason 1-3 (none in this study); group 2 is Gleason 4-6; group 3 is Gleason 7; and group 4 is Gleason 8-10. Unconditional logistic regression models are being used to assess the main effects of the genetic variants on occurrence of prostate cancer. Gene x gene interactions are analyzed by logistic regression using the Wald $\chi 2$ test to determine significant differences in slopes. Logistic regression for a polychotomous outcome is being used to assess associations with Gleason score ($\leq$6, 7, and $\geq$8). Gene x gene interactions for Gleason score are not being analyzed as there are too few individuals with Gleason scores 8-10 in order to reliably fit a model with interactions. Since the majority of the population was non-Hispanic white, adjustment for racial group is not being performed.

We are using a haplotype-tagging approach to examine the genetic variation in *SHBG, IRS1, IGFBP1*, *IGFBP3, IGF1* and *IGF1R*. This allows us to examine the genetic variation across the entire gene in order to not miss a possible association within the gene. There are additional steps to haplotype analysis that are not present in traditional, genotype-based case-control studies. First, a set of SNPs must be selected that will mark the common haplotypes in the population. These SNPs are commonly referred to as haplotype-tagging SNPs. The next step is the assignment of haplotypes to the case and control individuals, based on their haplotype-tagging SNP genotypes. Without genotype information in the parents or a direct molecular assay of individual chromosomes, the haplotypes must be assigned based on a probability model. We have developed algorithms for selecting haplotype-tagging SNPs and estimating haplotype assignments for the sampled individuals. The second algorithm assigns haplotypes to each individual, based on the individuals' genotype data and the estimated population haplotype frequencies. The output is a matrix with a column for each of the common haplotypes present in the study population (frequency greater than 0.05) and a row for each individual. A logistic regression can be carried out with the haplotype data to estimate the risk of disease associated with each haplotype.

Task 10: Months 26-36: Perform statistical analyses as outlined in Methods.

This task was completed in past years for *CYP17, IGF-1 str, CYP11A, VDR-BsmI, VDR-Taq1, VDR polyA, IRS1, SHBG,* and *SRD5A2-str*, the *INS, IGF1,* and *IRS2* variants with risk of prostate cancer and Gleason scores. Haplotype analysis also was previously reported for *SHBG* and *IGFBP1*.

Since the last annual report, we performed statistical analyses of the *PI3KCB, SHCI*, and *IGF1R* SNPs. There were two SNPs in *IGF1R* that showed significant associations with Gleason category. We are waiting to complete all the make-up genotyping prior to running the remaining analyses. We will then also explore gene x gene interactions for genes within the same pathway.

Task 11: Months 34-36: Prepare and submit final report and manuscripts.
Task 11 has not been started. A no-cost extension has been granted so that we can perform additional genotyping and analysis.

KEY RESEARCH ACCOMPLISHMENTS: The *IRS1* G972R GR/RR genotypes are associated with a 2.7-fold increased risk of prostate cancer risk and the *IRS2* G1057D GD/DD genotypes are significantly associated with cancer aggressiveness as measured by Gleason score.

REPORTABLE OUTCOMES: Our manuscript on our positive finding of *IRS1* was accepted and published during the previous annual report.

CONCLUSIONS: The *IRS1* G972R GR/RR genotypes are associated with a 2.7-fold increased risk of prostate cancer risk and the *IRS2* G1057D GD/DD genotypes are significantly associated with cancer aggressiveness as measured by Gleason score. These results provide additional support for an insulin-like growth factor and/or insulin pathway in the etiology of prostate cancer, and suggest that there are common variants associated with increases in prostate cancer risk and cancer aggressiveness. We continue to study additional genes in this pathway. Validation studies need to be performed to confirm these findings.

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APPENDICES: none